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Effects of *Azadirachta indica* Seed Extract on Larvae and Adult Anopheles Mosquitoes in Nasarawa Local Government Area, Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PSL, MDO, AE, JDCT and RJO conceived, designed and performed the experiment. Author OA contributed immensely in the search for the literature. All authors read and approved the final manuscript.

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ABSTRACT

Over the years, the use of agrochemicals has been one of the reasons for resistance of mosquitoes to insecticides, a negative trend that has been on the increase. This study was carried out to determine the effect of *Azadirachta indica* (neem) seed extract on the larvae and adult *Anopheles* mosquitoes in Nasarawa Local Government Area of Nasarawa state as a possible alternative to already existing insecticide against the parasite. The research was carried out at the insectary laboratory of Nasarawa state university Keffi. Impregnated papers of neem seed oil were used for adult *Anopheles* mosquitoes and the larvicidal effect was also determined. Adult *Anopheles* mosquitoes exposed to impregnated papers of neem seed extract at different concentrations of 20%, 40%, 80% and 100% showed 5%, 29%, 66% and 91% mortality rates respectively. Mortality rate was recorded at 24h of exposure with LC_{50} (Lethal concentration) of 4.45ml. The larvae were also exposed to different concentration of neem seed extract of 1%, 4%, 8% and 10% for 72h and the mortality rate was 100% with LC_{50} value (Lethal concentration) of 4.16ml. This implied that adult *Anopheles* mosquitoes will required more of the extract to achieve

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100% mortality than larvae. Using *Azadirachta indica* (neem) seed extract as an anti-mosquito agent was more effective on the larvae of Anopheles mosquitoes than the adult *Anopheles* mosquitoes.

Keywords: Anopheles mosquitoes; Azadirachta indica; lethal concentration; Nasarawa.

1. INTRODUCTION

"Malaria is one of the major causes of mortality and morbidity in Nigeria [1,2]. WHO has rated Nigeria 92nd in Africa where Nigeria accounted for 25% of the global burden" [3]. "Mosquitoes are not only the vectors of malaria but the vectors of several diseases" [4] "with *Anopheles gambiae* giles also known as Africa mosquito been the most common vector of human malaria in Afro Tropical Region" [5]. "In recent years, research has been going on where botanical metabolites are increasingly realized as potential substitute for chemical insecticides" [6].

"The neem plants belong to the family of Meliaceae plants which contain so many varieties of compound that shows insecticidal, antifeedant, growth-regulating, and development-modifying properties" [7-10]. *Melia azedarach* L. and *Azadirachtin indica* (Sapindales: Meliaceae), "commonly known as Chinaberry or Persian lilac tree, are deciduous trees that are native to north western India; and have long been recognized for their insecticidal properties. These trees grow typically in tropical and subtropical parts of Asia, but nowadays they are also cultivated in other warm regions of the world because of their considerable climatic tolerance. Fruit extracts of *Meliaazedarach and Azadirachta indica* elicit a variety of effects in insects such as anti feedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes of behavior" [11-15].

Previous studies [16,17] have shown that larvae of Anopheles mosquitoes exhibited a high mortality rate to seed oil and leaf extract but less mortality to the bark extract upon exposures at 12 hours.

Fathelrahman, [18] also observed "in Sinnar State -Sudan that *Anopheles arabiensis* were susceptible to the extract of leaf and seed of *Azadirachtin* with high susceptibility to the kernel seed".

"When applied to artificial water bodies in every two weeks within period of three months, emulsified neem oil have the same effect on larval mortality and adult density as the commonly synthetic insecticides used" [19]. "A study was also carried out using a neem oil formulation on third and fourth larvae stage of *Anopheles gambiae* and showed 50%inhibition of adult emergence at a concentration of 6ppm" [20]. "In a study using emulsified neem oil, it shows that within a three months, *Anopheles* larvae failed to develop resistance or change their susceptibility to the oil" [19].

This study therefore aimed at determing the effects of *Azadirachta indica* seed extract on Larvae and adult Anopheles mosquitoes in Nasarawa Local Government Area, Nasarawa State Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Nasarawa Local Government Area of Nasarawa state which is located in the western senatorial zone with latitude 08°41'20" to 08°32'10" N and longitude 07°48'43" to 07°50'00" E. It has two seasons rainy seasons starts from April to October while dry season start from October to March.

2.2 Sample Collection and Procedures

"The *Anopheles* mosquitoes larva were collected from their natural water habitat in some communities of Nasarawa Local Government Area using Entomological ladles into a well labelled container, they were transported the insectary laboratory, Department of Zoology Nasarawa State University Keffi. The *Anopheles* mosquitoes were reared to adult in a container in the laboratory, the larva were fed with biscuit and yeast while the adult were fed with sugar solution according to" WHO, [3].

2.3 Procedure for WHO Method for Susceptibility of Neem Oil Extract

"The susceptibility test was carried out using four tubes for each of the concentration (neem seed

extract) used with two control tubes each. An aspirator was used to introduce 25 Anopheles mosquitoes into the impregnated tubes. A timer was used to record both the number of dead and alive mosquitoes after 10 minutes for 1hour. After which the mosquitoes were transfer into a recovery tubes and were fed for 24 hour and the mortality rate was also recorded. The mortality in the control tubes was recorded as well" [3].

2.4 Procedure for Larviciding

Distilled water was introduced into four different containers for each of the concentration of the oil used with two controls. A pipette was used to introduce 25 Anopheles larvae into the containers and different concentration of neem seed oil was introduce into the containers. Mortality was recorded at 60 minutes, 24hour, 48hour, and 72hour. Mortality in the control was also recorded [3].

2.5 Data Analysis

The data were analyzed using descriptive statistics in percentages.

3. RESULTS

3.1 Effects of *Azadirachtin indica* Oil Extract on Adult Anopheles Mosquitoes

Result showed that a total of 400 adult *Anopheles* exposed to *A. indica* oil extract at different concentration for 24h. Mortality was recorded at 10 minutes, 60 minutes and 24h post exposure as shown in Table 1. The percentage of adult *Anopheles* mosquitoes to 20%, 40%, 80% and 100% concentration of *A. indica* oil extract at 60 mins of exposure were 0, 20, 47 and 49 percentage mortality. While the mortality recorded for 24h post exposure were 5, 29, 66 and 91 respectively.

3.2 The Larvicidal Effect of *A. indica* Oil Extract

The larvicidal effect of *A. indica* oil extract is as shown in Table 2 A total of 400 Anopheles larvae were exposed to different concentration of neem seed extract (1%, 4%, 8% and 10%) for 24h, 48h and 72h. The percentage mortality of neem seed extract to larvae showed 86.5%, 98.8% and 100% mortality. While 2(1%) mortality in the control was also recorded.

4. DISCUSSION

Adult *Anopheles* mosquitoes exposed to different concentration of neem seed oil at 20%, 40%, 80% and 100% for 60 mins and 24h post exposure mosquitoes were highly resistant at different dosage in all the concentration for 24h and at 100% they were less resistant (Table 1). This may be as a result of the effect of agricultural chemicals that were used to control pests in which the neem oil will not be effective enough in the knockdown. This present study shows that adult *Anopheles* mosquitoes were resistant to neem seed oil and in previous study in Sudan, Fatherahman [18] observed that A. *aranbiasis* were susceptible to neem seed oil.

Table 1. Effects of *Azadirachtin indica* oil extract on adult Anopheles mosquitoes

Concentration of oil extract (%)	No. of anopheles mosquitoes exposed	Mortality (%)	
		60(mins)	24h
20.0	100	0(0.0)	5(5.0)
40.0	100	20(20.0)	29(29.0)
80.0	100	47(47.0)	66(66.0)
100.0	100	49(49.0)	91(91.0)
Control R1	25	0(0.0)	1(0.25)
R2	25	0(0.0)	1(0.25)

Table 2. Larvicidal effect of *Azadirachta indica* seed oil extract

Concentration of oil extract (%)	No. of anopheles larval exposed	Mortality %		
		24h	48h	72h
1	100	78(88.0)	95(95.0)	100(100.0)
4	100	85(85.0)	100(100.0)	100(100.0)
8	100	88(88.0)	100(100.0)	100(100.0)
10	100	95(95.0)	100(100.0)	100(100.0)
Control R1	25	0(0.0)	0(0.0)	1(0.25)
R2	25	0(0.0)	0(0.0)	1(0.25)

Larval of *Anopheles* mosquitoes were exposed to different concentration of neem seed oil at 1%, 4%, 8% and 10% for 60 mins and 24h post exposure. In this research, the larval of *Anopheles* mosquitoes were susceptible to neem seed oil, this is because neem seed oil can cause deterrence in insect, repellency, growth disruption, reduced fitness and sterility activities (Table 2). This confirmed the previous study in Sokoto by Aleiro (2003) where larval of *Anopheles* mosquitoes were exposed to undiluted extract of neem seed oil and susceptibility was obtained. It was also observed by Adobu et al. [17] in Kogi State where larval of *Anopheles* mosquitoes were 100% susceptible to neem seed oil. (Kela et al. 2019) uses neem oil water dispersible tablet against the third instar larvae of Anopheles mosquitoes and its showed 98% mortality. Assalif et al. [21] also observed that neem powder applied on 1st, 2nd and 3rd instar larvae showed88.9%, 87.9% and 79.4% mortality. The mortality was very high at the first instar larval stage.

5. CONCLUSION

From this present study, neem seed extract showed efficacy as an anti-malaria agent by killing the mosquitoes subjected to it. Its efficacy was however more pronounced on the Anopheles larvae than the adult Anopheles mosquitoes. Therefore the use of neem seed extract should be encouraged in the control of mosquitoes but for a more appreciable result, the mosquito larvae should be targeted when using the neem seed extract.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Morakinyo OM, Balogun FM, Fagbamigbe AF. Housing type and risk of malaria among under-five children in Nigeria: evidence from the malaria indicator survey. Malaria Journal. 2018;(17):1-11.
2. World Health Organisation. World malaria report. World Health Organisation Geneva; 2018.
3. WHO. Test procedures for insecticides resistance in malaria vectors, Bio-effecacy and persistence of insecticides on treated surfaces. WHO/ CDS/CPC/Ma Journal. 1998;12: 98.
4. An NTG, Huong LT, Satyal PA, Tai T, Dai DN, Hung NH, Ngoc NTB, Setzer WN. Mosquito larviciding activity, antimicrobial activity and chemical compositions of essential oils from four species of myrtaceae from central Vietnam plants. 2020;(9):2-19.
5. Centre for Disease Prevention and Control. Anopheles mosquitoes, malaria; 2010. Available:https://www.cdc.gov/malaria/about/biology/#tabs-1-5 Accessed 14 june, 2019.
6. Vivekanandhan P, Venkatesan R, Ramkuma G, Karthi S, Senthil-Nathan S, Shivakumar MS. Comparative analysis of major mosquito vectors response to seed-derived essential oil and seed pod-derived extract from *Acacia nilotica*. International journal of environs resource and public health. 2018;15:1-10.
7. Nugroho BW, Edrada RA, Wray V, Witte L, Bringmann G, Gehling M, Proksch P. An insecticidal rocaglamide derivatives and related compounds from Aglaiaodorata (Meliaceae). Phytochemistry. 1999;51: 367–376.
8. Greger H, Pacher T. Brem B, Bacher, Mand-Hofer O. Flavaglines and other compounds from Fijian Aglaia species. Phytochemistry. 2001;57:57–64:1322 S.S.
9. D'Ambrosio M, Guerriero A. Degraded limonoids from *Melia azedarach* and biogenetic implications. Phytochemistry. 2002;60:419– 424.
10. Nakatani M, Abdelgaleil SAM, Saad MMG, Huang RC, Doe N, Iwagawa T. Phragmalinlimonoids from chukrasiatabularis. Phytochemistry. 2004; 65:2833–2841.
11. Schmidt GH, Rembold H, Ahmed AI, Breuer AM. Effect of *Melia azedarach* fruit extract on juvenile hormone titer and protein content in the hemolymph of two species of noctuid lepidopteran larvae (Insecta: Lepidoptera: Noctuidae). Phytoparasitica. 1998;26:283–291.
12. Abou Fakhr Hammad M, Zournajian Hand Talhouk S. Efficacy of extracts of MeliaazedarachL. callus, leaves and fruits

against adults of the sweet potato whitefly Bemisiatabaci (Homoptera: Aleyrodidae). Journal Applied Entomology. 2001;125:483–488.

13. Gajmer T, Singh R, Saini RK, Kalidhar SB. Effect of methanolic extracts of neem (*Azadirachta indica* A. Juss) and bakain (*Melia azedarach* L.) seeds on oviposition and egg hatching of Eariasvittella (Fab.) (Lepidoptera: Noctuidae). Journal of Applied. Entomolology. 2002;126:238–243.
14. Banchio E, Valladares G, Defago M, Palacios S, Carpinella C. Effects of *Melia azedarach* (Meliaceae) fruit extracts on the leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae): assessment in laboratory and field experiments. Ann. Appl. Biol. 2003;143:187–193.
15. Wandscheer CB, Duque JE, Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J, Fontana JD. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito Aedesaegypti. Toxicon. 2004;44:829–835.
16. Aliero BL. Larvaecidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of Anopheles mosquito in Africa Journal of Biotechnology. 2003;2:325-327.
17. Adobu US, Odoh CK, Akpi UK, Anya F. Activity of crude seed and leaf neem extracts (*Azadirachta indica*) against larvae in Kogi. American Journal of Micrology and Biotechnology. 2018;5(1):12-17.
18. Faltherahman IE. Effect of some neem (*Azadirachta indica*) Organic extracts against mosquitoes *Anopheles arabiensis* patton. Journal for Agricultural Sciences. 2017;32(2):128-134.
19. Awad OM, Shimaila A. Operational use of neem oil as an alternative Anophelinelarvicide, Part A: laboratory and field efficacy. East Mediterr Health Journal. 2003;9:637-645.
20. Okumu FO, Knols BG, Fillinger U. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. Malar Journal. 2007;6:63.
21. Assalif D, Meshesha B, Melaku G. Larvicidal activities of chinaberry, neem and *Bacillus thuringiensis* israelensi (Bti) to an insecticide resistant population of *Anopheles arabiensis* from Tolay, Southwest Ethiopia. Asian Pacific Journal of Tropical Biomedicine. 2016;6(7):554-561.

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